Treatment of IV in Boiling Water——A solution of IV (1.0 g) in H_2O (10 ml) was heated at 85—90° for 2 hr. The solution was evaporated *in vacuo* to give a pale yellow oily residue (0.9 g), which was subjected to column chromatography on a silicagel column. First elution with $CHCl_3$–MeOH (9: 1) gave 0.05 g of I (5.5%), second elution with $CHCl_3$–MeOH (9: 1) gave 0.45 g of V (56.8%) as a plae yellow oil. All compounds obtained here were identified by comparing their IR spectra with those of authentic samples.

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Studies of Oligosaccharides. XV.[1] Syntheses of Hydroquinone Glycosides of Gentio Oligosaccharides

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In the preceding papers of this series we have demonstrated the important role of sugar moieties in glycyrrhezin[3] and digitoxin[1] analogues, glycosides of pharmacological interests, as controllers of the hydrophilicity-lipophilicity balance of molecules, and also reported their effect on pharmacological activities as well as toxicities.[1] In continuation of these works this paper deals with the extension of the sugar part of arbutin by introduction of gentio oligosaccharides up to the tetraose to one of the hydroxyl groups in hydroquinone. Although the formation of the bioside from arbutin by enzymic transglycosylation was reported,[4] and the chromatographic evidences for the biosynthesis of this glycoside from hydroquinone and uridine diphosphate (UDP)-glucose are provided,[5,6] there appear to have been no papers on the chemical syntheses of this series of glycosides including the bioside.

The key intermediates of these glycoside syntheses, III and XI, were obtained by detritylation with 80% acetic acid at 70° of their precursors, II and X, respectively, which had been prepared by tritylation, followed by acetylation of I and VI, respectively.

The Königs-Knorr condensation of the intermediate III with acetobromo-glucose and -gentiobiose in the presence of silver oxide gave the acetates of the bioside and the trioside, respectively. Similarly the block condensation of XI with acetobromogentiobiose gave the acetate of the tetraoside. That the newly formed interglycosidic linkages were β was obvious from our extensive studies on the syntheses of these oligosaccharides.[7] Deacetylation of these acetates by the conventional method[8] afforded their parent glycosides, VI, IX and XIII.

1) Part XIV: K. Takiura, H. Yuki, Y. Okamoto, H. Takai, and S. Honda, *Chem. Pharm. Bull.* (Tokyo), **22**, 2263 (1974).
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3) K. Takiura, S. Honda, M. Yamamoto, H. Takai, M. Kii, and H. Yuki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1618 (1974).
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8) G. Zemplen, *Ber.*, **57**, 702 (1924).

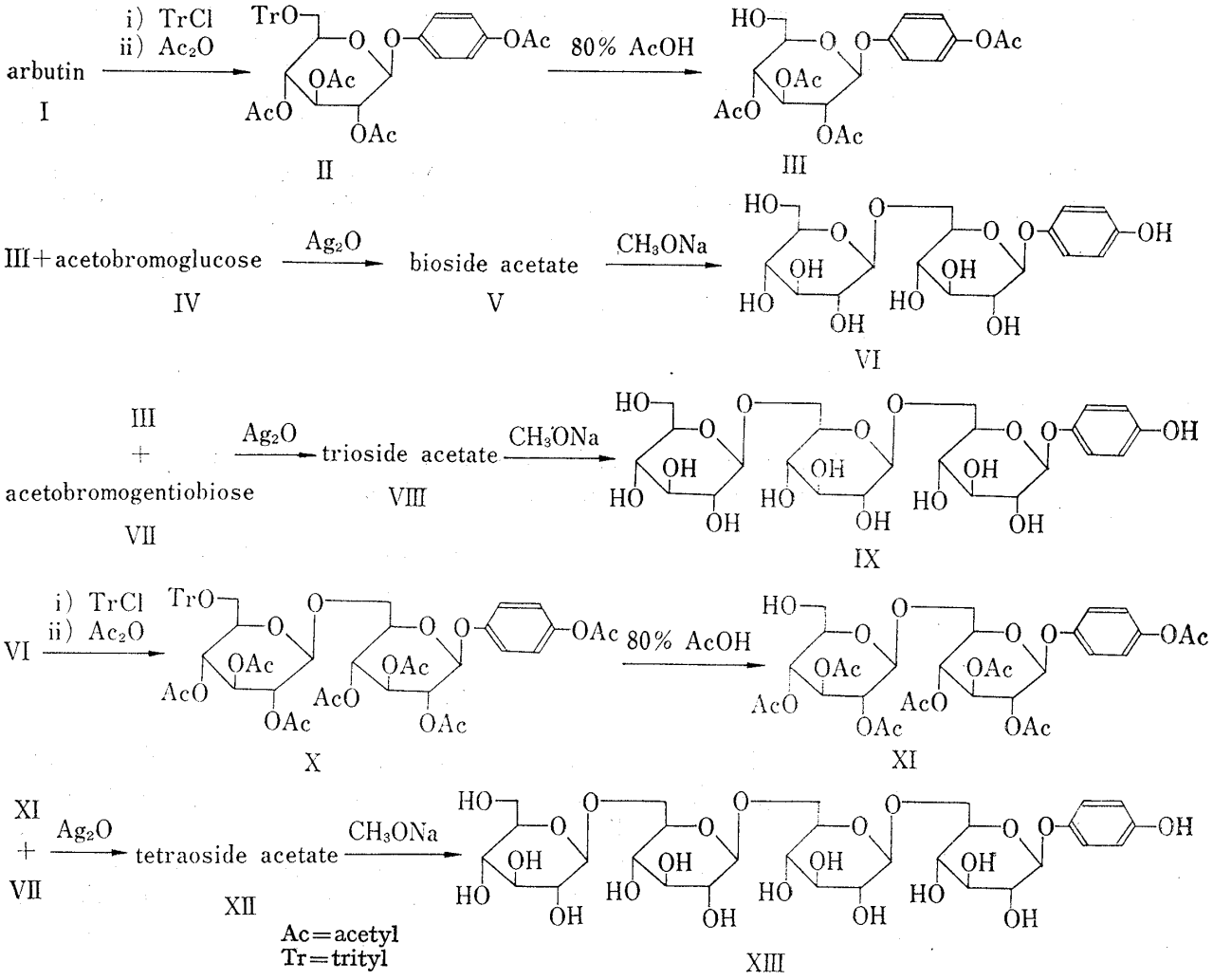

Chart 1

The synthetic analogues of arbutin thus obtained showed a linearity of log *Rf*/1-*Rf versus* the number of D-glucose residues[9] indicating that they belong to a homologous series.

The partition coefficients between water and *n*-butanol were determined from the ultraviolet (UV) absorption of both phases after equilibration. They conformed to the general rule established in our work of this series that the hydrophilicity increases with the increasing number of unit monosaccharides.[1,3]

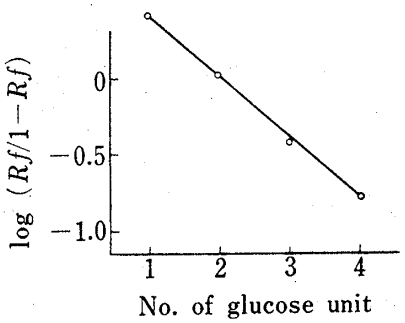

Fig. 1. Homology of Synthetic Analogues

solvent: 6: 4: 3 *n*-BuOH–pyridine–water

Table I. Water–Oil Distribution of Synthetic Analogues

	Partition coefficient water/*n*-BuOH (20°)
Hydroquinone	0.152
I	2.88
VI	16.4
IX	83.3
XIII	∞

9) D. French and G.M. Wild, *J. Am. Chem. Soc.*, 75, 2612 (1953).

The liberation of hydroquinone from these glycosides on the interaction of almond β-glucosidase was measured by paper chromatographic (PC) isolation, followed by UV absorption spectroscopy. The monoside (I) and the bioside (VI) were hydrolyzed nearly quantitatively within 3 hr, whereas glycosides with higher degree of polimerization (IX and XIII) gave rise to slower liberation of this phenol.

Bacteriostatic activity to *Staphylococcus aureus* was observed for I at concentrations higher than 10^{-3} M, whereas the other glycosides had no inhibitory effect even at the concentration of 10^{-2} M.

Experimental[10]

***p*-Acetoxyphenyl 2,3,4-Tri-O-acetyl-6-O-trityl-β-D-glucopyranoside (II)**——A mixture of arbutin (I, 60 g, 0.22 mole), trityl chloride (80 g, 0.29 mole) and dehydrated pyridine (600 ml) was stirred at 60° for 33 hr. The mixture was allowed to stand at room temperature for additional 60 hr, and then acetylated with Ac_2O (600 ml) at room temperature overnight. The reaction mixture was poured into ice water (10 kg) with vigorous stirring, and the precipitate was collected. Recrystallization from EtOH afforded needles (90 g, 60%), mp 202—205.5°; $[\alpha]_D^{23}$ +16.1° (c=3.73, $CHCl_3$). *Anal.* Calcd. for $C_{39}H_{36}O_{11}$: C, 68.81; H, 5.33. Found: C, 68.39, H, 5.46. IR ν_{max}^{KBr} cm^{-1}: 1500, 1600 (aromatic C=C), 1755 (CO).

***p*-Acetoxyphenyl 2,3,4-Tri-O-acetyl-β-D-glucopyranoside (III)**——A suspension of II (50 g) in 80% AcOH (1.5 liters) was heated at 70° with stirring. A complete solution resulted after 2 hr, which was evaporated to dryness. The residual syrup was dissolved in $CHCl_3$ (500 ml) and washed with water (500 ml), followed by satd. $NaHCO_3$ (500 ml). The $CHCl_3$ layer was dried on $CaCl_2$, and evaporated to dryness. The residual syrup was applied to a column of silica gel (Wakogel C-200, 5×40 cm). After elution of triphenylcarbinol with benzene, the detritylation product III (21 g, 68%) was eluted with EtOH, mp 149—154°; $[\alpha]_D^{24}$ −14.1° (c=1.57, $CHCl_3$). *Anal.* Calcd. for $C_{20}H_{24}O_{11}$: C, 54.54; H, 5.49. Found: C, 54.42; H, 5.55. IR ν_{max}^{KBr} cm^{-1}: 1500, 1595 (aromatic C=C), 1750 (CO), 3470 (OH).

***p*-Acetoxyphenyl β-Gentiobioside Heptaacetate (V)**——A mixture of III (6.1 g, 15 mmoles), Ag_2O (3.8 g), Drierite (15 g) and $CHCl_3$ (15 ml) was stirred at room temperature for 1 hr. Iodine (0.77 g) was added to this mixture, and subsequently a $CHCl_3$ solution (25 ml) of acetobromoglucose (IV, 6.1 g, 15 mmoles) was added dropwise with continuous stirring. After stirring overnight the reaction mixture was filtered and the precipitate washed with $CHCl_3$. The combined filtrate and the washing were evaporated to dryness, and the residual syrup was crystallized from MeOH to give the bioside acetate V (4.3 g, 37%), mp 222.5—223.5°; $[\alpha]_D^{31}$ −32.3° (c=3.76, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{42}O_{20}$: C, 52.99; H, 5.49. Found: C, 53.28; H, 5.48. IR ν_{max}^{KBr} cm^{-1}: 1500 (aromatic C=C), 1753 (CO).

***p*-Hydroxyphenyl β-Gentiobioside (VI)**——The acetate V (4.3 g) was suspended in MeOH (150 ml) and a minimum amount of $CHCl_3$ was added to effect complete dissolution. To this solution was added 0.1 M methanolic sodium methoxide (25 ml), and the mixture was stirred at room temperature for 4 hr. The reaction solution was deionized by stirring with Amberlite IR-120 (H^+) resin, decolorized with charcoal, and concentrated to 30 ml. A crystalline product of the bioside VI (1.8 g, 76%) separated after standing overnight, mp 265.5—267.5°; $[\alpha]_D^{22}$ −58.3° (c=0.75, H_2O). *Anal.* Calcd. for $C_{18}H_{26}O_{12}$: C, 49.77; H, 6.03. Found: C, 49.72; H, 6.01. UV $\lambda_{max}^{H_2O}$ nm(ε): 283.5 (2000). IR ν_{max}^{KBr} cm^{-1}: 1510, 1607 (aromatic C=C), 3220, 3400 (OH).

***p*-Acetoxyphenyl β-Gentiotrioside Decaacetate (VIII)**——This compound was obtained from the reaction of III (9.1 g, 21 mmoles), Ag_2O (5.3 g), Drierite (21 g), iodine (1.1 g), VII (14.7 g, 21 mmoles) and $CHCl_3$ (50 ml) for 4 days in a similar manner as described for the preparation of V. Yield, 13.0 g (59%). Recrystallization of the product from EtOH, followed by MeOH, afforded needles, mp 241—242°; $[\alpha]_D^{26}$ −24.3° (c=2.88, $CHCl_3$). *Anal.* Calcd. for $C_{46}H_{58}O_{28}$: C, 52.18; H, 5.52. Found: C, 52.09; H, 5.58. IR ν_{max}^{KBr} cm^{-1}: 1502 (aromatic C=C), 1760 (CO).

***p*-Hydroxyphenyl β-Gentiotrioside (IX)**——Deacetylation of VIII in a similar manner as described for the deacetylation of V afforded the trioside IX in a yield of 78%, mp 205—207°; $[\alpha]_D^{21}$ −59.5° (c=1.71,

10) All evaporations were carried out below 40° under diminished pressure. Melting points were determined on a hot stage with a Yanagimoto micro melting point apparatus and are uncorrected. Specific rotations were measured in a 1-dm tube. UV and IR absorption spectra were obtained with Hitachi ESP-3T and EPI-G2 spectrometers. UV determination was performed with a Hitachi EPU-2A spectrophotometer. Descending PC was carried out on Whatman No. 1 filter paper with 6:4:3 *n*-BuOH–pyridine–water unless otherwise mentioned. Spots of phenolic glycosides were visualized with alkaline silver nitrate[11] and diazotized *p*-nitroaniline.[12]

11) W.E. Trevelyan, D.P. Procter, and J.S. Harrison, *Nature*, **166**, 444 (1950).

12) J.B. Pridham, *Anal. Chem.*, **29**, 1167 (1957).

H_2O). *Anal.* Calcd. for $C_{24}H_{36}O_{17}$: C, 48.32; H, 6.08. Found: C, 48.52; H, 6.64. UV $\lambda_{max}^{H_2O}$ nm(ε): 283.5 (1900). IR ν_{max}^{KBr} cm^{-1}: 1505 (aromatic C=C), 3370 (OH).

***p*-Acetoxyphenyl 2,3,4,2′,3′,4′-Hexa-O-acetyl-6′-O-trityl-β-D-gentiobioside (X)**——The bioside VI (4.5 g, 10.8 mmoles) and trityl chloride (3.0 g, 10.8 mmoles) were dissolved in dehydrated pyridine (90 ml) and the solution was heated at 80° for 21 hr. Trityl chloride (6.1 g, 21.8 mmoles) was supplemented, and the reaction mixture was heated for additional 29 hr. After cooling Ac_2O (90 ml) was added and the mixture was allowed to stand overnight. The reaction mixture was poured into ice water (5 kg) and the precipitate was collected. Removal of contaminant triphenylcarbinol by silica gel column chromatography in a similar manner as described for the purification of III from its crude product, followed by recrystallization from EtOH afforded the refined product of X (2.8 g, 26%), mp 117—123°; $[\alpha]_D^{21}$ −9.7° (c=2.63, $CHCl_3$). *Anal.* Calcd. for $C_{51}H_{54}O_{19}$: C, 63.09; H, 5.61. Found: C, 62.65; H, 5.90. IR ν_{max}^{KBr} cm^{-1}: 1498 (aromatic C=C), 1775 (CO).

***p*-Acetoxyphenyl 2,3,4,2′,3′,4′-Hexa-O-acetyl-β-gentiobioside (XI)**——Detritylation of X in a similar manner as described for the detritylation of II gave compound XI in a yield of 73%. Recrystallization from EtOH afforded needles, mp 200—202°; $[\alpha]_D^{20}$ −42.7° (c=2.86, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{40}O_{19}$: C, 52.75; H, 5.53. Found: C, 52.54; H, 5.71. IR ν_{max}^{KBr} cm^{-1}: 1498 (aromatic C=C), 1750 (CO), 3450 (OH).

***p*-Hydroxyphenyl β-Gentiotetraoside (XIII)**——The crude tetraoside acetate XII was obtained as a syrup from the reaction of XI (1.5 g, 2.1 mmoles), Ag_2O (0.52 g), Drierite (2.1 g), iodine (0.10 g), VII (1.5 g, 2.2 mmoles) and $CHCl_3$ (5 ml) for 20 days in a similar manner as described for the preparation of V. Yield, 3.0 g. TLC (Wakogel B-5, 19:1 $CHCl_3$–MeOH, detected with concentrated sulfuric acid) examination of the crude product indicated the presence of a main spot having *Rf* 0.40 along with several minor spots. The Crude acetate was deacetylated, without purification by crystallization, in a similar manner as described for the deacetylation of V. The syrupy crude product of XIII was applied to a charcoal column (charcoal for chromatography, Nishio Kogyo Co., Ltd., 2×42 cm), and the column was eluted stepwise with water (500 ml), 5% EtOH (800 ml) and 20% EtOH (2 liters) in this order. From the 20% EtOH fraction the tetraoside XIII (0.38 g, 24%) was obtained after evaporation of solvent, which was further purified by preparative PC on Whatman No. 3MM filter paper with 6:4:3 *n*-BuOH–pyridine–water. The refined product of XIII was obtained as amorphous powder which gave a single spot on PC. UV $\lambda_{max}^{H_2O}$ nm(ε): 283.5 (1600). IR ν_{max}^{KBr} cm^{-1}: 1505 (aromatic C=C), 3400 (OH). A part of XIII (37 mg) was acetylated by the conventional method with a 1:1 Ac_2O–pyridine mixture to give its acetate (XII, 50 mg, 80%), mp 147—152°; $[\alpha]_D^{17}$ −49° (c=0.57, H_2O). *Anal.* Calcd. for $C_{58}H_{74}O_{36}$: C, 51.71; H, 5.54. Found: C, 51.76; H, 5.70. *Rf* (TLC) 0.40.

Water/*n*-BuOH Partition Coefficients——Arbutin analogues (1—2 mg) were dissolved in water-satd. *n*-BuOH (5.00 ml) and to these solutions were added *n*-BuOH-satd. water (5.00 ml). The mixtures were shaken constantly on a temperature-controlled water bath for 24 hr, and then allowed to stand overnight. The aqueous layers were diluted with water to appropriate volumes, and the absorbancies were read at 283.5 nm except for hydroquinone (289.5 nm). Concentrations in aqueous phases were obtained from these values against calibration curves, and concentrations in organic phases were calculated from those in aqueous phases and the amounts of samples used. The partition coefficients, tabulated in Table I, were obtained as ratios of concentrations in both phases.

Enzymic Hydrolysis——To 0.1M solutions of arbutin analogues were added β-glucosidase (10 mg, Sigma Co., Ltd., lot 125B-0100, prepared from almond) and the mixtures were incubated at 37°. Aliquots of 15 μl were applied on Whatman No. 1 filter paper and developed with 4:1:5 *n*-BuOH–AcOH–water (upper layer). Spots of hydroquinone were monitored by a UV lamp, and extracted with MeOH (10 ml). From absorbancies at 294.5 nm the amounts of hydroquinone liberated during incubation were calculated.

Bacteriostatic Activity——Solutions of four different concentrations (10^{-5}, 10^{-4}, 10^{-3} and 10^{-2}M) of each arbutin analogue were prepared. To each of these solutions was added a pepton culture (9 ml, pH 6.8) containing meat extract (50 mg), pepton (10 mg) and NaCl (50 mg). After inoculation with *Staphylococcus aureus* 209P, each mixture was incubated at 37° for 24 hr. Minimum bacteriostatic concentrations were obtained by comparing the turbidity of sample solutions with that of controlls.